Results after replantation of avulsed permanent teeth. II. Periodontal healing and the role of physiologic storage and antiresorptive-regenerative therapy

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Abstract – The status of the periodontal ligament (PDL) and of the pulp are decisive for the healing of avulsed and replanted teeth. A tooth rescue box was developed and distributed to offer optimal storage conditions for avulsed teeth. The therapy comprised extraoral endodontic treatment and applications of medicaments to enhance periodontal healing. In this long-term clinical study the healing results following avulsion and replantation were investigated. Twenty-eight permanent teeth in 24 patients were evaluated. The extraoral storage media and periods varied considerably. Soon after avulsion six teeth were stored in a cell culture medium (tooth rescue box Dentosafe[®]) for 1–53 h; the PDL was defined as not compromised. Sixteen teeth were stored in a non-physiologic situation temporarily, the PDL was considered as compromised. Six teeth were stored in non-physiologic conditions for longer periods; the condition of the PDL was defined as hopeless. On 14 teeth antiresorptive-regenerative therapy (ART) with the local application of glucocorticoids and enamel matrix derivative and the systemic administration of doxycyclin was used. In all teeth extraoral endodontic treatment by retrograde insertion of posts was performed. The mean observation period was 31.2 months $(\pm 24.1; 5.1-100.2;$ median: 23.8). All six teeth rescued physiologically healed with a functional PDL (functional healing, FH) irrespective of the storage period. Of eight teeth with a compromised PDL on which ART was used, three teeth healed with a functional PDL. All other teeth showed replacement resorption, in three teeth additionally infection-related resorption was recorded. The predominant influence on the healing results was the immediate physiologic rescue of avulsed teeth (chi-square, P = 0.0001). The use of ART seemed to support FH (chi-square, P = 0.0547) in teeth with a compromised PDL. No other factors (maturity of roots, crown fractures, gender, age, antibiotics) were related to healing. In a linear regression analysis physiologic storage (P = 0.0013) and ART (P = 0.0421) were related to FH. For a good prognosis avulsed teeth should be stored immediately

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in a cell-compatible medium. Therefore the tooth rescue box should be distributed at places with higher risks of accidents, in families with children and in emergency facilities. The use of ART might have potential for enhancing the prognosis of teeth with a PDL that is compromised but not in a hopeless condition. Extraoral endodontic treatment reduces infection-related complications; this item was discussed in the first part of the publication.

Avulsion is a serious injury to teeth. Pulp and periodontal ligament (PDL) tissues are injured; besides, dental hard tissues, alveolar bone and gingiva might be affected. The vessels and nerves of the pulp rupture at the apical foramen, the pulp tissues undergo necrosis. Damage of different origin will result in destroyed tissues and cell death in the PDL. The basic healing processes following plantation are commonly accepted (1–5). Cells with clastic activity resorb the damaged tissues, eventually including the cementum and dentin. In some minorly damaged regions the ruptured peridontal fibers are just rebuilt, in histologic preparations no differences can be recognized compared with a sound PDL (regeneration). When resorption cavities are small, neighboring cementoblasts recolonize the denuded root surface and deposit the cementum in which newly formed periodontal fibers are anchored. This so-called healing-related resorption (formerly named surface resorption) and regeneration represent healing with physiologic function (functional healing, FH). Larger resorption cavities may not be completely covered by the cementoblasts in time. Regenerating alveolar bone comes into contact with the root surface and through physiologic bone remodeling the dentin and cementum are consecutively replaced by bone (replacement resorption/ankylosis, RR). When an establishing endodontic infection is not or not adequately treated infection-related resorption (IRR, formerly named inflammatory resorption) will result. Subtypes of IRR were defined in the first part of the present publication according to timing of occurrence and etiology (6). It was differentiated between an early IRR (e-IRR), a tunneling IRR succeeding a partial RR (t-IRR) and a cervical resorption succeeding a complete RR (cRR-CR).

Dry storage of isolated teeth rapidly leads to cell death in the PDL adhering to the root (for overview, see 7). This process is slowed down by storage in certain media (milk, saline, saliva) but progressive and inevitable and therefore the storage in these conditions is limited to short periods (8–17). Special cell culture media maintain vitality and proliferative capacity of PDL cells for extended periods (15, 18–20). In a clinical study the extraoral condition (immediate replantation, duration of dry and wet storage) as well as the maturity of the roots were significantly related to periodontal healing in multivariate analysis (21).

Limited storage in isotonic solutions prior to plantation of teeth with a moderate damage to the PDL was shown to give equal (22) or even better (7, 23, 24) healing results compared with teeth planted immediately. It was concluded that the interim storage washes off microorganisms and toxic substances deriving from autolysed cells (23, 24). The storage in cell-compatible culture media is said to have a certain reconditioning effect on damaged PDL cells; in animal experiments storage in special cell culture media following a certain dry phase improved periodontal healing after replantation of extracted teeth (20).

In animal experiments tetracycline decreased root resorption when applied topically (25) or systemically (26, 27). This was also demonstrated for glucocorticoids when applied topically but not when administered systemically in animals (28).

Enamel matrix derivative (EMD) (Emdogain®; Biora, Malmö, Sweden) was introduced to promote periodontal regeneration (29). In animal experiments extracted teeth were stored dry for 30 min and, after application of EMD, were replanted into their sockets (30) or transplanted to the abdominal wall (31). Compared with teeth planted without EMD histologic results exhibited less root resorption, better healing of resorption cavities and promoted periodontal healing. In a clinical study ankylosed teeth were therapeutically replanted after application of EMD onto the root surface and into the alveolus. During a mean observation period of 15 months a reestablishment of the ankylosis was not observed in teeth that had been ankylosed due to a less severe trauma (32, 33).

A tooth rescue box (Dentosafe®; Dentosafe GmbH, Iserlohn, Germany; EMT Tooth Saver, SmartPractice.com, Phoenix, AZ, USA) was introduced (19, 34) and distributed in schools in parts of Germany and Switzerland and in all schools in Austria. The tooth rescue box contains a tissue culture medium similar to a medium used during islet cell transplantation. Besides different salts the medium also contains amino acids, vitamins, and glucosis. The medium was shown to maintain vitality and proliferative capacity of PDL cells for up to 48 h at room temperature in vitro (18, 19). Due to an added protect medium and a preservative the unopened box has a shelf life of 3 years at room temperature (below 37°C).

The purpose of the study was to evaluate the healing results of avulsed and replanted permanent teeth and to identify parameters that influence healing. Factors already tested in the first part of the present study were also considered (6).

Material and methods

Since 1975 avulsed teeth with predictable pulp necrosis were treated following a standard protocol which was adapted to actual knowledge and enhanced possibilities in 1995 and 1998. The basis was and is immediate extraoral endodontic treatment by retrograde insertion of posts with diameters of up to 3 mm, adapted to the diameter of the root (1, 6, 35, 36). Up to 1995, during the extraoral phase, the teeth were kept moist by and stored in sterile physiologic saline. Since 1995 they were stored in the tissue culture medium of the tooth rescue box (Fig. 1). Since 1998 additional treatments were used to depress resorption activities and enhance periodontal regeneration.

Immediately after arrival of the patient the avulsed teeth were stored in the named media. A history was taken comprising the duration and the media of extraoral storage. The patient was thoroughly examined clinically and radiographically (extraoral and intraoral radiographs). Before 1995 already during this examination extraoral endodontic treatment was prepared and started by a second oral surgeon when pulp necrosis was predictable according to the maturity of the root and/or the circumstances during the extraoral phase. Thus replantation was completed within 30-45 min after arrival of the patient. Since 1995 the teeth were stored at least 30 min in the tissue culture medium; replantation as soon as possible was no longer followed. Every few minutes the teeth were shaken gently within the medium. Before 1998 systemic antibiotics (mostly penicillin)



Fig. 1. Avulsed tooth rescued in tooth rescue box.

were prescribed but not on a regular basis. Since 1998 doxycyclin for systemic use was always prescribed as part of antiresorptive-regenerative therapy (ART).

Extraoral endodontic treatment

According to the method described by Kirschner et al. and Kirschner (1, 35, 36) the avulsed teeth were endodontically treated by an extraoral retrograde insertion of posts made of ceramics (Cerasiv, Plochingen, Germany; no longer available) or titanium (RetroPost®; Komet/Gebr. Brasseler, Lemgo, Germany). The method is described in detail in the first part of the present publication (6).

Intensive rinsing

For rinsing exclusively sterile isotonic saline was used. The root surface of the avulsed teeth was intensively rinsed throughout the preparation of the root canal by the automatic cooling system. Up to 1998 the root surface was additionally rinsed immediately before replantation. Since 1998 the root surface was rinsed as soon as possible after arrival of the patient, during the preparation of the root canal and immediately before the application of a glucocorticoid to the storage medium (see later). The alveolus was rinsed also immediately before replantation.

Antiresorptive-regenerative therapy

Since 1998 the topical and systemic application of different medicaments was used to depress resorption activity and support regeneration in the PDL. After completion of the extraoral endodontic treatment and intense rinsing of the root surface the teeth were stored for about 20 min in the tissue culture medium of the tooth rescue box Dentosafe[®] to which 40 μg ml⁻¹ dexamethasone had been added. Every few minutes the teeth were shaken gently within this medium. Immediately before replantation - after removal of the coagulum from the alveolus by rinsing with sterile isotonic saline – an EMD (Emdogain®) was applied onto the root surface and into the alveolus. After completion of the treatment doxycyclin was prescribed for systemic use according to the patients' weight (2 mg kg⁻¹ body weight daily, maximum dose 100 mg day⁻¹) for 5 days.

Additional remarks

Macroscopic visible contamination of the teeth was removed by an intense and sharp flow of saline. In

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single cases larger attached pieces of dirt were carefully removed with small forceps. In no case the root surface was rubbed or otherwise touched. However, contamination or success of cleansing were not recorded.

In no case the root surface was chemically treated (i.e. application of fluoride). Fractures or infractions of the alveolus were not regularly noted.

Immediately before replantation the blood clot in the alveolus was carefully removed, and the alveolus was intensively rinsed with sterile isotonic saline.

Classification of postoperative healing

According to clinical and radiographic examinations the healing was classified as FH, RR or IRR. Subtypes of IRR were classified as e-IRR, t-IRR, and cRR-CR according to the different etiology and timing of occurrence as proposed in the first part of the present study (6).

Results

All 28 avulsed and replanted teeth were evaluated (6). The mean age of the 24 patients was 10.3 years (±2.6, 7.1–17.3, median: 9.7). Except one lateral upper incisor exclusively central upper incisors were involved. Data for the individual teeth are given in Table 1.

The extraoral dry time was 3–150 min. Following the initial dry storage 16 teeth were stored in wet but non-physiologic conditions (saline, milk, Ringer) for 30–120 min, eight teeth were stored in physiologic conditions (tooth rescue box) for 30–3180 min. Following the interim storage in non-physiologic media nine teeth were stored in the tooth rescue box for 55–2460 min. In the treatment phase nine teeth were stored in saline, 19 teeth were stored in the medium of the tooth rescue box. The exact duration of the storage during the treatment phase was not recorded and was set to 45 min for the statistics. This represents typical values but may differ for about 15 min.

In total, non-physiologic storage duration (rescue and treatment phase) was 3–255 min, the physiologic storage duration (medium of the tooth rescue box) was 45–3225 min (0.75–53.75 h). The total extraoral time varied between 85 and 3235 min (1.4–53.9 h).

Nine teeth exhibited FH while 19 teeth showed RR. Of these 19 teeth three exhibited radiolucent areas in the cervical region (two teeth) or along the post (one tooth). The diagnosis was set to IRR, subtypes were cRR-CR and t-IRR. No early IRR was recorded (6). The mean observation period was 31.2 months (±24.1; 5.1–100.2; median: 23.8). The mean observation period for teeth exhibiting FH

was shorter than that for teeth showing healing complications (23.1 and 35.0 months).

Ten teeth were replanted without an interim storage in the medium of the tooth rescue box or the use of ART (nothing). Four teeth were replanted after an interim storage in the medium of the tooth rescue box without the use of ART (box). Fourteen teeth were replanted after interim storage in the medium of the tooth rescue box and with the use of ART

For further analysis the condition of the PDL at the time of placement in the tooth rescue box was defined as not compromised, when the teeth were stored dry for less than 15 min and/or stored in a non-physiologic wet medium for less than 30 min. The PDL was classified as *compromised* when the dry storage was between 15 and 60 min and/or the non-physiologic wet storage was between 30 and 120 min. The condition of the PDL was defined hopeless when the extraoral time exceeded 60 min of dry and/or 120 min of non-physiologic wet storage. Thus six teeth were classified as not compromised, 16 as compromised and six as hopeless (Fig. 2). The used classification showed a high and significant correlation with the healing results (chi-square, P = 0.0002).

All six teeth with a not compromised PDL (= immediate physiologic storage) showed FH, on four of them ART was used. The storage duration in the medium of the rescue box varied between 1 and 53 h.

Six teeth were stored in milk following an initial dry phase. In four cases the dry phase was up to 15 min and the storage in milk was 30–60 min. One tooth exhibited FH, two teeth showed RR and one tooth cRR-CR (Fig. 2).

Of the 16 teeth with a PDL defined as compromised 13 teeth showed complications and three teeth demonstrated FH. On eight of these 16 teeth ART was used. Five of these eight teeth showed RR while three teeth exhibited FH. Teeth showing IRR were not treated by ART. The non-physiologic extraoral phase of teeth treated with ART did not exceed 90 min of dry and 120 min of non-physiologic wet storage.

In the chi-square test immediate physiologic storage was closely related to periodontal healing (all cases included, FH vs. complication: P = 0.0001, FH vs. RR vs. IRR: P = 0.0003). The influence of ART on periodontal healing was at the borderline of significance (FH vs. complication, all cases: P = 0.0430, compromised PDL: P = 0.0547). All other factors (maturity of roots, crown fractures, gender, age, use of antibiotics) were not related to periodontal healing (chi-square, P > 0.1) (Table 2).

All parameters that showed a significant relationship to healing in the chi-square tests

Table 1. Avulsion of permanent incisors in growing patients and replantation following immediate extraoral endodontic treatment (retrograde insertion of posts). Patient-, rescue- and treatment related factors and healing results

							Res	Rescue		Treatment	Total e.	Total extraoral storage	eßi					
No.	Patient	Age (years)	Tooth	Antib	Crown frac.	Dry (min)	Medium wet	Unphys wet (min)	Box (min)	[Medium] (45 min)	Unphys wet total (min)	Box total (min)	Extraoral total (min)	PDL cond.	ART	Healing	Observ. (months)	Removal
-	-	13.1	Ξ	No	No	09	Box	0	150	Box	0	195	255	ပ	Box	RR	22.0	×
2	2	8.3	Ξ	Yes	Dentin	9	Milk	09	0	Box	09	45	115	ပ	ART	RH	31.7	In situ
က	က	10.0	21	Yes	9	က	Вох	0	09	Box	0	105	108	2	Box	func	62.1	In situ
4	4	10.9	Ξ	Yes	9	40	Nein	0	0	Saline	45	0	82	ပ	N	RR	100.2	In situ
2	2	9.5	Ξ	Yes	Dentin	30	Saline	30	0	Saline	75	0	09	ပ	9	RR	30.9	×
9	2	9.2	7	Yes	Dentin	30	Saline	30	0	Saline	75	0	09	ပ	٩	RB	30.9	In situ
7	9	8.8	7	٩	Dentin	15	Ringer	09	0	Box	09	45	120	ပ	Box	t-IRR	44.6	×
8	7	12.3	Ξ	Yes	S	09	Saline	120	2460	Вох	120	2505	2685	ပ	ART	RR	22.6	In situ
6	80	7.7	Ξ	Yes	9	15	Milk	09	720	Вох	09	765	840	ပ	ART	func	17.8	In situ
10	œ	7.7	7	Yes	Enamel	15	Milk	09	720	Box	09	292	840	ပ	ART	RB	17.8	In situ
Ξ	6	11.2	Ξ	٩	2	120	Nein	0	0	Saline	45	0	165	ᅩ	٩	RB	24.1	×
12	10	7.9	Ξ	Yes	9	45	Вох	0	30	Вох	0	75	120	ပ	ART	func	9.9	In situ
13	Ξ	10.9	Ξ	Yes	Dentin	09	Saline	45	840	Вох	45	885	066	ပ	ART	func	21.3	In situ
14	12	9.0	7	Yes	Dentin	65	Nein	0	0	Saline	45	0	110	ᅩ	8	RH	9.68	×
15	13	12.1	7	Yes	Pulp	15	Saline	30	840	Box	30	885	930	2	ART	func	37.1	In situ
16	14	12.6	7	Yes	9	150	Saline	09	0	Saline	105	0	255	ᅩ	٩	cRR-CR	72.5	In situ
17	15	9.1	7	Yes	2	09	Вох	0	720	Box	0	765	825	ပ	Box	RB	25.2	In situ
18	16	12.2	7	Yes	9	9	Вох	0	3180	Box	0	3225	3235	2	ART	func	16.6	In situ
19	17	8.5	7	No	Enamel	30	Ringer	09	0	Saline	105	0	06	ပ	N N	RH	53.4	×
20	92	6.6	Ξ	Yes	Dentin	9	Milk	30	0	Saline	75	0	82	ပ	S	cRR-CR	31.5	×
21	19	8.0	Ξ	Yes	9	6	Milk	30	1290	Box	30	1335	1455	ᅩ	ART	RH	5.1	×
22	19	8.0	7	Yes	2	6	Milk	30	1290	Вох	30	1335	1455	ᆮ	ART	HH.	5.1	×
23	20	17.3	7	Yes	2	9	Plast. foil	06	22	Вох	06	100	200	ပ	ART	RH	23.4	In situ
24	7	9.7	Ξ	Yes	9	2	Box	0	210	Box	0	255	260	2	8	func	12.4	In situ
22	22	7.1	Ξ	N	2	61	Desinf.	45	0	Saline	06	0	151	ᆮ	8	HH.	10.3	In situ
56	23	7.9	7	Yes	2	09	Saline	120	720	Box	120	765	945	ပ	ART	HH.	24.1	×
27	54	13.3	Ξ	Yes	Dentin	က	Box	0	09	Box	0	105	105	2	ART	func	16.9	In situ
28	54	13.3	7	Yes	N	က	Вох	0	09	Вох	0	105	105	20	ART	func	16.9	In situ

PDL cond., PDL condition; nc, not compromised; c, compromised; h, hopeless. ART, antiresorptive-regenerative therapy; func, functional healing; RR, replacement resorption; t-IRR, tunneling infection-related resorption; cervical resorption. X, extraction/removal.

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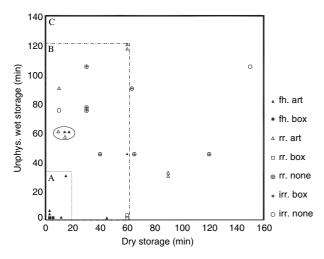


Fig. 2. Scatterplot of healing results after avulsion and replantation following extraoral insertion of posts in relation to duration of extraoral dry and unphysiologic wet storage. fh, functional healing (filled symbols); rr, replacement resorption; irr, infection-related resorption. Oval marking: storage in milk. A: defined as not compromised. B: defined as compromised. C: defined as hopeless.

[post material (6), storage, ART] were subjected to a linear regression analysis for all data. Immediate physiologic rescue (P = 0.0013) and ART (P = 0.0421) were factors with a significant influence on healing (FH vs. complication).

Discussion

The prognosis of avulsed and replanted teeth is dependent on the periodontal healing which is influenced by the condition of the PDL tissues. Damage of the PDL of avulsed teeth may derive from direct mechanical trauma (contusion between tooth and bone), from the storage conditions (dry, non-physiologic media), from the non-optimal situation after replantation due to supply and disposal exclusively by diffusion, from attached microorgan-

isms or other contaminations and from toxic substances released from autolysed cells.

The prerequisite for FH is the absence of infection. Early infection-related complications derive from an infected pulp space and – if untreated – will result in early tooth loss. As an important part of our concept in the treatment of isolated teeth with predictable pulp necrosis extraoral endodontic treatment with the retrograde insertion of posts is used to prevent an endodontic infection and therefore to minimize IRR. This was discussed in the first part of the present publication (6).

Besides the secure and successful endodontic treatment our actual concept concentrated on the prevention of RR. This comprised the physiologic rescue of avulsed teeth, the washing off of toxic substances and bacteria by intensive and repeated rinsing of the root surface, the reconditioning of PDL cells by interim storage in a cell-compatible medium and an attempt of antiresorptive, antiosteo-blastic and PDL regeneration promoting therapy (ART). Short and non-rigid splinting was a prerequisite.

The predominant influence on PDL healing was the cell-compatible storage in the tooth rescue box soon after avulsion of a tooth. All of the six teeth rescued within 3–15 min exhibited FH free of complications, irrespective of the storage duration that was up to 53 h. This is in accordance with results from *in vitro* investigations on this medium: as demonstrated by autoradiography and in cell cultures the medium maintained vitality and proliferative capacity of PDL cells (cementoblasts, fibroblasts) at room temperature for at least 48 h (18, 19). Comparable media gave similar results in animal experiments (15, 20).

According to the successful 'long-term' storage it seems recommendable to temporarily store teeth rescued in non-physiologic conditions in an optimal cell-compatible medium to use a possible recondi-

Table 2. Cross tabulation of all recorded parameters vs. healing results. P-values of chi-square and linear regression statistics

	FH - complication		FH-RR-IRR	
	All data $(n = 28)$	Compromised $(n = 16)$	All data $(n = 28)$	Compromised $(n = 16)$
Chi-square				
Post material	0.0122		0.0319	
Diameter, sealer, surgeon, surgical experience	n.s.		n.s.	
Phys-unphys storage	0.0002		0.0003	
ART - no ART	0.0430	0.0547	0.0491	0.0784
Maturity	n.s.		n.s.	
Age, gender, tooth position, antibiosis, crown fractures	n.s.		n.s.	
Regression				
Phys-unphys storage	0.0013		0.0005	
ART - no ART	0.0421		0.0950	
Post material	0.5982		0.7987	

FH, functional healing; RR, replacement resorption; IRR, infection-related resorption; phys, physiologic storage; unphys, unphysiologic storage; ART, antiresorptive-regenerative therapy.

tioning effect before the cells are brought into the non-optimal conditions of a coagulum and a nutrition situation that is restricted to diffusion. To avoid a diffusion barrier during the extraoral storage the teeth should gently be shaken every few minutes within the medium. This storage method also contributes to washing off bacteria and toxic breakdown products from cell death which lead to worse results when left (7, 18, 23, 24). Repeated rinsing of the root surface during extraoral endodontic treatment might wash off more intensively these deleterious metabolites and microorganisms than passive soaking, thus enhancing healing. All three teeth with a compromised PDL that were stored in the medium of the tooth rescue box – and without the use of ART - exhibited non-functional healing. Thus a 'reconditioning effect' (7, 18, 23, 24) could not be demonstrated with the present material. However, the non-physiologic periods of the cases in the present study were much longer (i.e. 60 min dry) than in other studies (20–40 min dry). After 60 min dry storage most cells are not only damaged but non-vital, and therefore they cannot be recovered.

The use of ART comprises a combination of different treatment strategies. The local application of a glucocorticoid decreased the resorptive activity in an animal experiment and enhanced periodontal healing (28). This has also been shown for the systemic and local application of tetracyclines in animals (25, 26). With the use of EMD less resorptions and better periodontal healing were observed in planted teeth in animal experiments (30, 31). The combined use of these substances has not been described to date. It is not known whether or not there might be synergistic effects - or the contrary. At least no healing complications were observed in the present study when physiologically rescued teeth were treated with this approach.

Except two cases the non-physiologic extraoral phase of teeth treated with ART did not exceed 60 min of dry and 120 min of non-physiologic wet storage. A good distribution of the tooth rescue box in the surroundings of our clinic obviously resulted in a rescue in physiologic conditions following a limited non-physiologic storage. Therefore, data on the usability of ART in cases with a definitely nonvital PDL are rare. According to the mechanisms of glucocorticoides and doxycyclin the establishment of a functional periodontium is impossible when all specialized cells are non-vital. Although the use of EMD reduced resorptions in replanted teeth and enhanced PDL healing (30, 31) it has not been shown to date that EMD could induce a functional periodontium in situations with a complete non-vital PDL. When damaged and ankylosed teeth were

extracted and replanted after extraoral insertion of posts and application of EMD teeth with minor traumas and therefore supposingly minor ankylotic areas regained a functional PDL while teeth with a severely damaged PDL reankylosed after some months (32, 33). Obviously a minimal population of vital specialized PDL cells, i.e. cementoblasts and fibroblasts, is a prerequisite for FH. A differentiation or a differentiation in sufficient numbers of pluripotent precursor cells toward cementoblasts cannot be expected in severely traumatized teeth by used medicaments. The use of ART in teeth with a completely non-vital PDL is experimental and will not be successful in supporting FH: despite the use of ART in teeth which were stored for more than 60 min dry and/or non-physiologically wet all these teeth ankylosed.

In some cases teeth with extended but limited non-physiologic extraoral storage (compromised PDL) exhibited FH when ART was used. Most probably a sufficient number of cementoblasts and fibroblasts had survived. Healing was supported by ART according to the chi-square test. However, the case number is low and the storage conditions of the individual teeth showed great variations. Nevertheless, the use of ART in these cases may be helpful.

Of the six teeth rescued immediately in physiologic conditions two teeth were replanted without, four teeth were replanted with ART. None of these teeth ankylosed. From these few cases it cannot be decided whether or not ART should be used in cases with physiologic rescue.

Healing following interim storage in media such as milk, saline and salt solution (Ringer) was not predictable even when ART was used. It was disappointing that even relatively short storages in milk for up to 1 h and following a relatively short dry phase resulted in RR in three of four cases. Media like milk or saline should not be considered 'physiologic'; death of PDL cells stored in these media is slowed down compared with dry storage but progressive and inevitable (8–17).

In a clinical study immature teeth showed significantly better 'periodontal healing' (=FH) than mature teeth. However, the incidence of IRR was higher in immature teeth (21, 37). In the present study no correlation could be found between the maturity of roots and FH. This may be explained by the lower case number and a negative preselection: Until recently, immature teeth were only subjected to the described treatment (i.e. extraoral endodontic treatment) – and therefore included into the present study – when they were rescued in a non-physiologic way. Otherwise they were replanted without endodontic treatment expecting revascularization. However,

we decided to also treat immature teeth that were rescued in a physiologic way by immediate extraoral insertion of posts to prevent any endodonticrelated complications (6). In the present study the rate of IRR was equally low in mature and immature teeth that is in contrast to a previous study (37). This can be explained by the different endodontic treatment approaches (6).

No relations to healing were found for gender, age, antibiotics, and crown fractures. This is in close correlation with published findings (21). However, in the present study a (single) case with pulp exposure due to crown fracture was included while in another study these complications were excluded (37).

The existence of vital PDL cells with proliferative capacity is decisive for periodontal healing following replantation. The use of the tooth rescue box containing a special cell culture medium has proved successful in storing avulsed teeth for up to 53 h. This enables delayed replantations in cases of more serious injuries (polytrauma) which require immediate medical care to prevent and treat situations endangering life. The box contains a preservative to minimize bacterial growth. However, a rescued tooth should be put into a new tooth rescue box after about 24 h when longer storage periods are necessary. Avulsed teeth need not be replanted as soon as possible after the arrival of the patient at the dentist. Careful examinations, including radiographs, can be carried out. The replantation procedure can even be planned according to patient's and dentist's needs. Data are scarce as regards whether there are minimum or maximum storage periods and whether a certain storage period would give superior results. Easily available the tooth rescue box can also be used during interventions such as intentional replantation or transplantation of teeth.

It is of interest that all teeth were rescued within some minutes in the tooth rescue box when it was available near the accident. The simple purchase of the box obviously had a high remembrance and educational potential. This resulted in immediate and correct reaction without any training of the lay persons. No telephone calls to dentists were necessary. Thus valuable minutes could be spared. The probability of FH was just 45% when mature teeth were replanted between 5 and 10 min after avulsion and decreased rapidly with time (21). Furthermore, teeth are usually not replanted at the site of the accident (38). It is important to distribute tooth rescue boxes at locations with a high risk of traumatic tooth injuries (schools, kindergartens, public pools, sporting facilities), at emergency units (ambulances, hospitals, physicians, dentists) and in families with children.

Conclusion

All teeth rescued in physiologic conditions shortly after avulsion healed in with physiologic function. Extraoral endodontic treatment and the use of ART (topically Glucocorticoides and Emdogain®, systemically Doxycyclin[®]) did not result in complications in these cases. There are hints that ART might promote healing in teeth with an extraoral nonphysiologic storage of limited duration. Immediate physiologic storage of avulsed teeth is of utmost importance for periodontal healing following replantation. The tooth rescue box should be widely available in first aid kits in schools, kindergartens, public pools, sporting facilities as well as in emergency facilities, pharmacies, at physicians and dentists and in families with children to allow a short reaction time after avulsion which is crucial for successful replantation.

References

- Kirschner H, Pohl Y, Filippi A, Ebeleseder K. Unfallverletzungen der Zähne. Vorbeugen - Retten - Behandeln, 1st edn. Hannover: Schlütersche; 2002.
- Andreasen JO, Andreasen FM, Bakland LK, Flores MT. Traumatic dental injuries. A manual, 1st edn. Copenhagen: Munksgaard; 2000.
- Tsukiboshi M. Treatment planning for traumatized teeth, 1st edn. Berlin: Quintessenz; 1999.
- Curzon MEJ. Dental trauma, 1st edn. Oxford: Wright; 1999.
- Andreasen JO, Andreasen FM. Textbook and color atlas of traumatic injuries to the teeth 3rd edn. Copenhagen: Munksgaard; 1994.
- Pohl Y, Filippi A, Kirschner H. Results after replantation of avulsed permanent teeth. I. Endodontic considerations. Dent Traumatol 2005;21:80–92.
- Andreasen JO, Paulsen HU, Zhijie Y, Schwartz O. A longterm study of 370 autotransplanted premolars. Part III. Periodontal healing subsequent to transplantation. Eur J Orthod 1990;12:25–37.
- Blomlöf L, Otteskog P. Viability of human periodontal ligament cells after storage of monkey teeth in milk or saliva. Scand J Dent Res 1980;88:436–40.
- Blomlöf L. Storage of human periodontal ligament cells in a combination of different media. Acta Odontol Scand 1981;60:1904–6.
- Doyle DL, Dumsha TC, Sydiskis RJ. Effect of soaking in Hank's balanced salt solution or milk on PDL cell viability of dry stored human teeth. Endod Dent Traumatol 1998;14:221-4.
- Hiltz J, Trope M. Vitality of human lip fibroblasts in milk, Hanks balanced salt solution and Viaspan storage media. Endod Dent Traumatol 1991;7:69–72.
- 12. Huang S-C, Remeikis NA, Daniel JC. Effects of long-term exposure of human periodontal ligament cells to milk and other solutions. J Endod 1996;22:30–3.
- Lekic PC, Kenny DJ, Barrett EJ. The influence of storage conditions on the clonogenic capacity of periodontal ligament cells: implications for tooth replantation. Int Endod J 1998;31:137–40.
- 14. Oikarinen K, Seppa ST. Effect of preservation media on proliferation and collagen biosynthesis of periodontal ligament fibroblasts. Endod Dent Traumatol 1987;3:95–9.

- Trope M, Friedman S. Periodontal healing of replanted dog teeth stored in Viaspan, milk and Hank's balanced salt solution. Endod Dent Traumatol 1992;8:183–8.
- Zimmermann M, Willershausen-Zönnchen B. In-vitro-Studie zur Überlebensquote humaner Desmodontalfibroblasten in verschiedenen Medien. Z Zahnärztl Implantol 1990;4:201–4.
- Pongsiri S, Schlegel D, Zimmermann M. Überlebensrate desmodontaler Zellen nach extraoraler Lagerung in verschiedenen Medien. Dtsch Z Mund Kiefer Gesichtschir 1990;14:364–8.
- Pohl Y, Kirschner H. Autoradiographische Untersuchungen zur Erfassung von Zellneubildung im Restdesmodont isolierter Zähne des Menschen. Dtsch Z Mund Kiefer Gesichtschir 1994;18:224–7.
- Pohl Y, Tekin U, Boll M, Filippi A, Kirschner H. Investigations on a cell culture medium for storage and transportation of avulsed teeth. Aust Endod J 1999;25:70–
- 20. Pettiette M, Hupp J, Mesaros S, Trope M. Periodontal healing of extracted dogs' teeth air-dried for extended periods and soaked in various media. Endod Dent Traumatol 1997;13:113–8.
- 21. Andreasen JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors. 4. Factors related to periodontal ligament healing. Endod Dent Traumatol 1995;11:76–89.
- 22. Andreasen JO, Schwartz O. The effect of saline storage before replantation upon dry damage upon the periodontal ligament. Endod Dent Traumatol 1986;2:67–70.
- 23. Cvek M, Granath LE, Hollender L. Treatment of non-vital permanent incisors with calcium hydroxide. III. Variation of occurrence of ankylosis of reimplanted teeth with duration of extra-alveolar period and storage environment. Odontol Revy 1974;25:43–56.
- 24. Matsson L, Andreasen JO, Cvek M, Granath L. Ankylosis of experimentally reimplanted teeth related to extra-alveolar period and storage environment. Pediatr Dent 1982;4:327.
- Cvek M, Cleaton JP, Austin J, Lownie J, Kling M, Fatti P. Effect of topical application of doxycycline on pulp revascularization and periodontal healing in reimplanted monkey incisors. Endod Dent Traumatol 1990;6: 170–6.

- Sae-Lim V, Wang CY, Choi GW, Trope M. The effect of systemic tetracycline on resorption of dried replanted dogs' teeth. Endod Dent Traumatol 1998;14:127–32.
- Sae-Lim V, Wang CY, Trope M. Effect of systemic tetracycline and amoxicillin on inflammatory root resorption of replanted dogs' teeth. Endod Dent Traumatol 1998;14:216–20.
- Sae-Lim V, Metzger Z, Trope M. Local dexamethasone improves periodontal healing of replanted dogs' teeth. Endod Dent Traumatol 1998;14:232–6.
- Hammarström LE. Enamel matrix, cementum development and regeneration. J Clin Periodontol 1997;24:658

 –68.
- Iqbal MK, Bamaas N. Effect of enamel matrix derivative (EMDOGAIN) upon periodontal healing after replantation of permanent incisors in Beagle dogs. Dent Traumatol 2001;17:36–45.
- 31. Hamamoto Y, Kawasaki N, Jarnbring F, Hammarstrom L. Effects and distribution of the enamel matrix derivative Emdogain in the periodontal tissues of rat molars transplanted to the abdominal wall. Dent Traumatol 2002;18:12–23.
- 32. Filippi A, Pohl Y, von Arx T. Treatment of replacement resorption with Emdogain preliminary results after 10 months. Dent Traumatol 2001;17:134–8.
- 33. Filippi A, Pohl Y, von Arx T. Treatment of replacement resorption with Emdogain[®] − a prospective clinical study. Dent Traumatol 2002;18:138−43.
- Kirschner H, Burkard W, Pfütz E, Pohl Y, Obijou C. Frontzahntrauma. Aufbewahrung und Behandlung des verunfallten Zahnes. Schweiz Monatsschr Zahnmed 1992;102:209–14.
- Kirschner H, Bolz U, Enomoto S, Hüttemann RW, Meinel W, Sturm J. Eine neue Methode kombinierter autoalloplastischer Zahnreplantation mit partieller Al2-O3-Keramikwurzel. Dtsch Zahnärztl Z 1978;33:594–8.
- 36. Kirschner H. Atlas der chirurgischen Zahnerhaltung, 2nd edn. München: Hanser; 1996.
- Andreasen JO, Borum MK, Jacobsen HL, Andreasen FM. Replantation of 400 avulsed permanent incisors. 1. Diagnoses of healing complications. Endod Dent Traumatol 1995:11:51–8.
- 38. Trope M. Clinical management of the avulsed tooth: present strategies and future directions. Dent Traumatol 2002;18:1–11.